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Introduction

Fundamental research clearly shows that the product of the c-myc oncogene is often expressed at elevated levels in a large number of breast cancers and is associated with poor prognosis, high-risk disease (for further detail, see our recent review (3). Indeed, Myc drives cell proliferation and can initiate as well as contribute to tumor development, as confirmed by both in vitro and in vivo models of breast cancer. The highly conserved regions of the Myc protein are thought to participate in protein:protein interactions. Importantly, blocking these sites can effectively inhibit Myc function and block breast carcinoma cell growth. Thus, Myc is a valid target for the development of novel therapeutics to inhibit mammary cells of malignant transformations in a specific and sensitive manner. This is the primary goal of this proposal.

To achieve our goal, we are using the novel Repressed Transactivator (RTA) assay developed in collaboration with our lab (1). The RTA is an in vivo functional assay that will enable Myc-binding proteins and inhibitors of Myc:protein interactions to be identified and characterized. This experimental tool is conceptually similar to the conventional "two-hybrid" technology but offers significant improvement. First, the RTA allows transactivator bait proteins like Myc, to be used in a two-hybrid approach to clone novel interactors. Second, the ability of the RTA to identify inhibitors of a given protein:protein interaction makes it particularly well suited for high throughput screening of protein:protein interactions and the identification of inhibitor compounds.

Objective/hypothesis

Our hypothesis is that Myc is a valid target for the development of novel therapeutics aimed at blocking essential Myc:protein complexes that drive the carcinogenic process of mammary epithelial cells.

Specific Aims

a. We will use the RTA to identify the Myc-binding domain of a recently identified Myc-interactor, TRRAP (2), and delineate the nature of a "Myc-signature sequence" with which other Myc-binding proteins can be identified by sequence databank analysis.
b. Clone and characterize novel Myc-binding protein using the RTA to screen for MycNTD interactors in cells derived from human breast carcinomas.
c. With these experimental tools in hand we will then use the RTA to identify specific inhibitors of these protein:protein interactions that will block Myc function and inhibit tumor cell proliferation.

Body

With support from the DOD, the research outlined in the original proposal has progressed in a steady and productive manner for the first two years of funding. To delineate the accomplishments to date, the tasks outlined in the original Statement of Work are itemized below and a progress report for each task provided.

Statement of Work

Task 1. To identify the Myc binding domain of TRRAP, a novel MycNTD-binding protein recently identified biochemically (months 1-18)

a. Complete. We have cloned overlapping fragments of TRRAP into the pBD prey vector using a PCR based approach and verify sequence is in-frame with the TUP-1 repressor domain.

b. Complete. The yeast have been transformed with these TRRAP fusion proteins, and analyzed by western blot for equal expression of prey fusion proteins using our recently derived TUP-1 specific antibody.
c. Complete. It has been established that the prey fusion proteins do not affect yeast viability in the presence or absence of 5-FOA.
d. Complete. We have tested TRRAP fusion proteins for their interaction with wildtype MycNTD bait proteins, include MbI and MBII deletion and point mutants as well as specificity controls for MycNTD interaction, including Gal4E2F, Gal4VP16, etc.
e. Complete. The region of TRRAP required for interaction with Myc has been further refined using smaller fragments of TRRAP in the RTA (Figure 1). To further resolve the binding sites we employed smaller fragments of TRRAP and surveyed a panel of Myc NTD point mutants, but were unable to conclusively identify the key amino acid contact points. However, we are presently conducting Nuclear Magnetic Resonance (NMR) studies, in collaboration with Dr. Cheryl Arrowsmith, using the smaller TRRAP fragment (F6) and Myc NTD regions identified by RTA. Once this is complete the required residues will be mutated and further evaluated in vivo.
f. In progress. Search the sequence database for other polypeptides encoding this motif
g. Yet to do. If such proteins are identified, their cDNA will be cloned and tested for Myc interaction using the RTA.
h. Yet to do. Additional proteins identified by this approach will be further characterized as stated in Task 2g-k.

Task 2. To use the RTA to identify MycNTD-binding proteins that are essential for Myc to transform mammary epithelial cells (months 1-24)

a. Complete. The cDNA libraries have both been prepared in pBD prey vector with mRNA derived first from asynchronous MCF-7 cells and then from primary breast carcinoma samples. The former was constructed as a directional library while the latter was prepared as a non-directional library.
b. Complete. Both cDNA libraries have been screened such that all mRNA in the cells is represented in the library screen. The MCF-7 library is superior to the primary breast library and will be used for subsequent analyses.
c. Complete. Pick and streak resultant colonies and retest for specificity of interaction on selection medium +/- 5-FOA +/- methionine to induce prey expression as well as a master plate to retain yeast for future work (see Figure 2).
d. Complete. Those colonies that show interaction with MycNTD in a prey fusion specific manner will be processed further. To isolate prey, high-quality plasmid DNA will be isolated by Qiagen or Clonetech prep and then transformed by electroporation into electro-competent DH5alpha cells.
e. Complete. Resultant mini-prep prey vector DNA will be retested for functional interaction against the MycNTD using the RTA.
f. Complete. Those that repeat will be tested for interaction with MycNTD containing deletion and point mutations in MbI and MbII.
g. Complete. Those that are dependent upon these highly conserved regions will undergo nucleotide sequencing.
h. Complete. These will be analyzed for known and novel cDNAs and interaction further confirmed in vitro by GST fusions, in vivo by co-immunoprecipitation of ectopically

expressed and tagged proteins and finally by in vivo co-immunoprecipitation of endogenous proteins.

i. In progress. Ectopic expression of the tagged protein will be analyzed by immunofluorescence to determine subcellular localization relative to Myc. For the latter excellent antibody reagents are available.

j. In progress. Myc-binding proteins will be tested for their ability to alter Myc function. These assays will include Myc autosuppression, apoptosis, transformation, etc. These assays are well-established in the lab.

k. In progress. The expression of Myc and the Myc-binding proteins will be analyzed for expression and activity in cell lines and primary tissue samples derived from normal and transformed mammary carcinomas.

Task 3. To identify functional inhibitors of Myc by targeting and blocking MycNTD interaction with key cellular binding proteins (months 18-36)

a *Yet to do. Establish MycNTD and novel interactor fused to TUP1 in yeast strain designed for the inhibitor screen. In this system, interaction causes death of yeast upon selection. Peptide aptamer combinatorial libraries are introduced into yeast and rapidly screened by selection for rescue of yeast cell death.

b *Yet to do. Peptide is isolated, and retested for functional inhibition of MycNTD and the Myc-binding protein.

c *Yet to do. These experiments are difficult to describe without the molecules in hand. For example, if the Myc-binding protein is known to bind other cellular proteins then it will be determined whether the peptide binds in a similar or unique manner than the natural cellular blocking protein. By this approach the mechanism of inhibition of the peptide can be determined. Moreover, specificity of interaction can be evaluated first in the RTA system against other cellular molecules with similar structure or function, and then in mammalian cells

d *Yet to do. Assays to measure the efficacy, specificity and sensitivity of peptide inhibition of Myc and the Myc-binding protein interaction as well as Myc function will be tested in mammalian cells.

*The RTA system can work well as a screening system to identify inhibitors of protein:protein interactions, as proposed. However, evaluating this system to screen for peptides that can block Myc:TRRAP interaction, with the minimal regions of these proteins that interact, has resulted in an assay with a poor signal to noise ratio. The high background precludes its utility as a screening tool for this pair of interactors. To address the goals of Task3, we have adopted an alternative approach that has been successful and will enable the objectives of Task 3 to be accomplished. Our approach is outlined below.

Task 3. To identify functional inhibitors of Myc by targeting and blocking MycNTD interaction with key cellular binding proteins (months 18-36)

a. In progress. To identify the contact points between TRRAP and Myc, we have initiated structural studies to analyze the smaller TRRAP and Myc NTD fragments identified by RTA (Task 1, Part e). This work will employ various structural and protein biochemistry techniques including NMR spectroscopy, CD spectroscopy and gel filtration, which will be conducted in collaboration with Dr. Cheryl Arrowsmith. The preliminary results show that the TRRAP:Myc interaction detected by RTA can also be detected by NMR

and CD spectroscopy. Experiments are currently being conducted resolve the exact amino acid contact points in the complex.

b. Yet to do. When the solution structure of the TRRAP:Myc complex is solved, we will use this model as a guide to generate non-functional mutants of TRRAP and Myc that are interaction defective. These mutants would be highly informative and invaluable tools for the study of TRRAP:Myc specific activities in vivo.
c. Yet to do. The detectable binding of the TRRAP and Myc NTD fragments by NMR will provides a useful system to monitor either small molecular weight compounds or peptide inhibitors of this interaction. If such molecules are identified by this approach, their use as inhibitors in vivo will be further tested using cell-based assays.

Key Research Accomplishments

•A region of TRRAP essential for interaction with Myc has been identified using the RTA. This Myc-binding domain of TRRAP and the Myc N-terminal domain are now under further analysis to define the precise amino acids required for this interaction (Figure A1)

•Two cDNA libraries have been constructed and screened to identify polypeptides that interact with the Myc N-terminus. Positive clones from the MCF-7 library have been isolated, re-screened and sequenced.

•Positive clones from the MCF-7 library screen have been tested for interaction with Myc in vitro. Select full-length clones have been generated and are now being tested for interaction with Myc in vivo under both exogenous and endogenous protein expression conditions (Figures B1-B18).

Reportable Outcomes

- Abstract presented at Reasons for Hope, Breast Cancer Conference, Orlando 2002
- Ph.D. degree conferred based on project supported by this award
- Employment opportunities received based on training supported by this award

Conclusions

The work to date shows the expected progress and suggests the ultimate goal of the proposal will be accomplished during the tenure of this award. Significant progress has been made in the second year of funding. Specific objectives outlined in task #1 and #2 are nearly complete.

As described in the first specific aim, the RTA has been employed to identify a region of TRRAP which is required for binding to the Myc N-terminal domain. As outlined, partial fragments of TRRAP and Myc were tested by RTA to isolate the regions within each protein that are critical for interaction. Identification of these smaller regions has enabled us to embark on NMR studies to identify the exact amino acid contact point between Myc and TRRAP. This work is presently in progress. Once binding requirements of the TRRAP:Myc interaction have been defined, inhibitors of this interaction will be identified (Task 3). While our technical approach has changed from the initial proposal, our aim to find novel therapeutics that specifically abrogate Myc function in transformation remains the same. Indeed, the combination of RTA and NMR technologies is a highly efficient approach to address this goal. While we have exploited the TRRAP:Myc interaction in this pilot project, we expect to extend this strategy to the other Myc interactions isolated in Task 2. The lead compounds generated by this work will have the potential to be further developed into novel therapeutics that block Myc function as an oncogene.

As outlined in the second specific aim, two cDNA libraries have been constructed and screened using the RTA to identify novel Myc binding proteins. Positive clones resulting from the MCF-7 library screen are presently being further characterized to ensure they are true Myc interactors and to reveal their identity through nucleotide sequencing. With these interactors in hand the molecular mechanism of action of Myc in the carcinogenic process will be further revealed. In addition, these clones will provide the essential experimental tools to develop additional therapeutics that will break Myc interactions that hold a key role in the transformation.

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2. McMahon, S. B., Van Buskirk, H. A., Dugan, K. A., Copeland, T. D., and Cole, M. D. (1998) The novel ATM-related protein TRRAP is an essential cofactor for the c- Myc and E2F oncoproteins. *Cell* 94, 363-74

3. Oster, S. K., Ho, C. S., Soucie, E. L., and Penn, L. Z. (2002) The myc oncogene: MarvelouslY Complex. *Adv Cancer Res* 84, 81-154

Appendices

Appendix A:
Figure A1

Appendix B:
Figure B1-B18

APPENDIX A

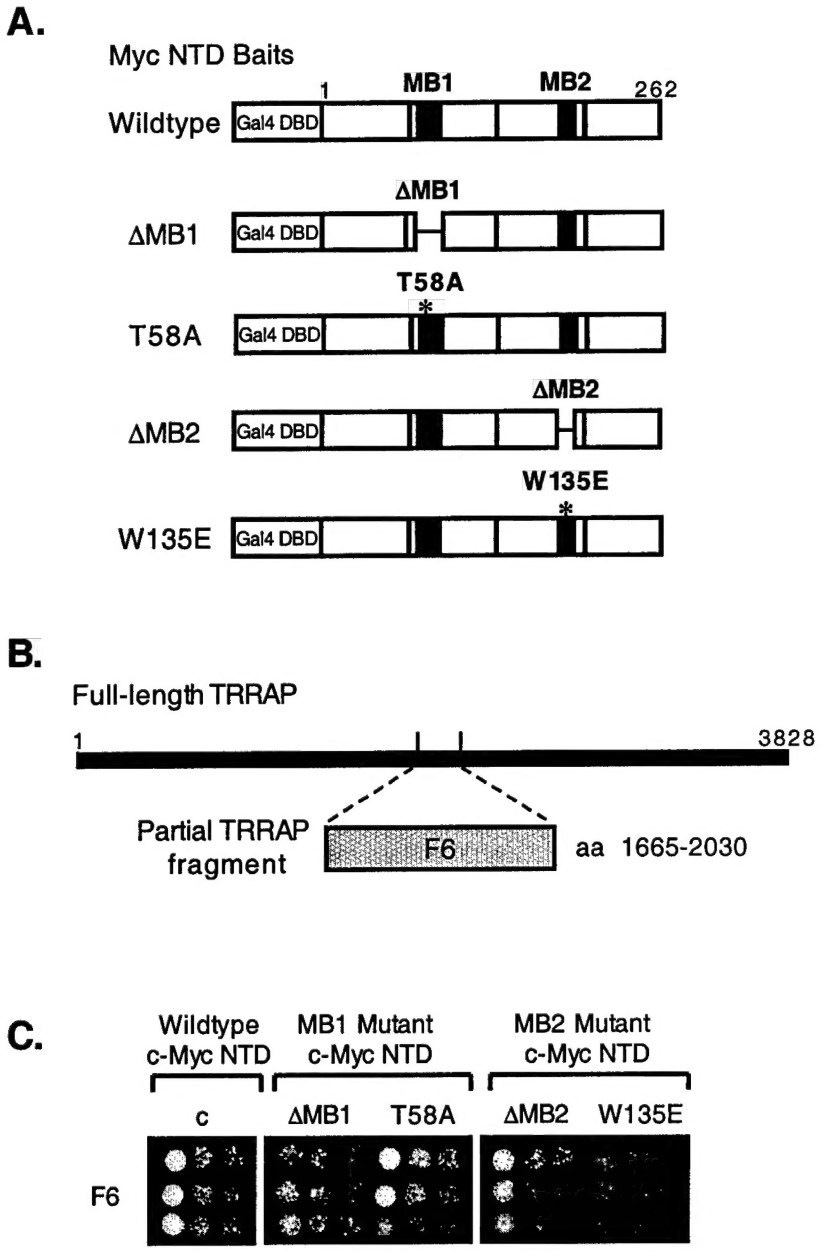


Figure A1. Mapping of the TRRAP interaction site within the Myc NTD
(A) Wildtype Gal4-Myc NTD and mutant Gal4-Myc NTD baits. Mutant baits include Myc Box (MB)1 and MB2 deletion mutants (ΔMB1and ΔMB2) and T58A and W135E point mutants. **(B)** Partial TRRAP F6 fragment. **(C)** Deletion of MB1 (ΔMB1) or MB2 (ΔMB2) and mutation of a conserved T58 phosphorylation site (T58A) within MB1, had no significant effect on Myc NTD and TRRAP F6 interaction in this system, whereas point mutation at W135 (W135E) significantly disrupted the interaction leading to loss of yeast growth on FOA selection.

APPENDIX B

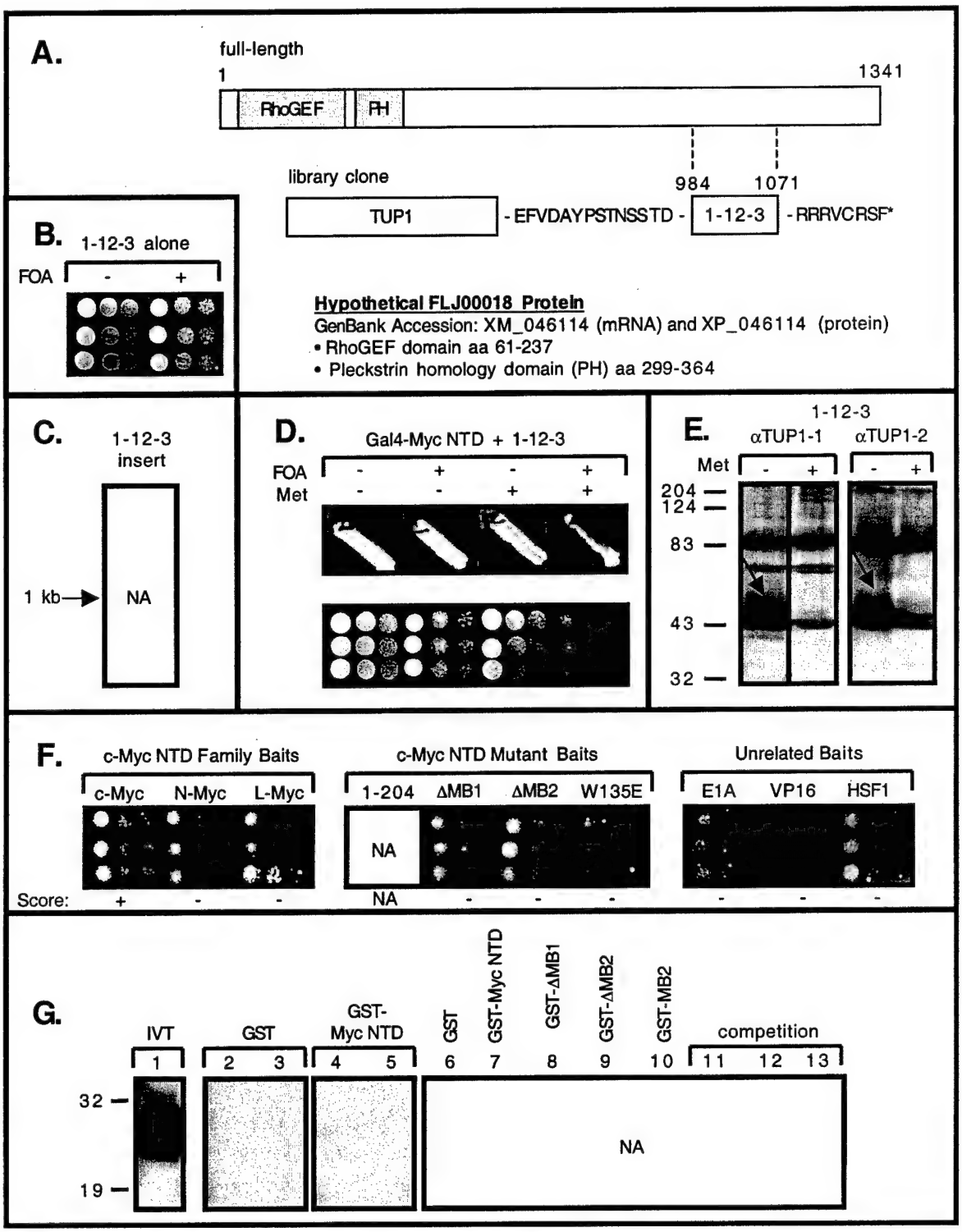


Figure B1. Library clone 1-12-3, Hypothetical FLJ00018 Protein

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

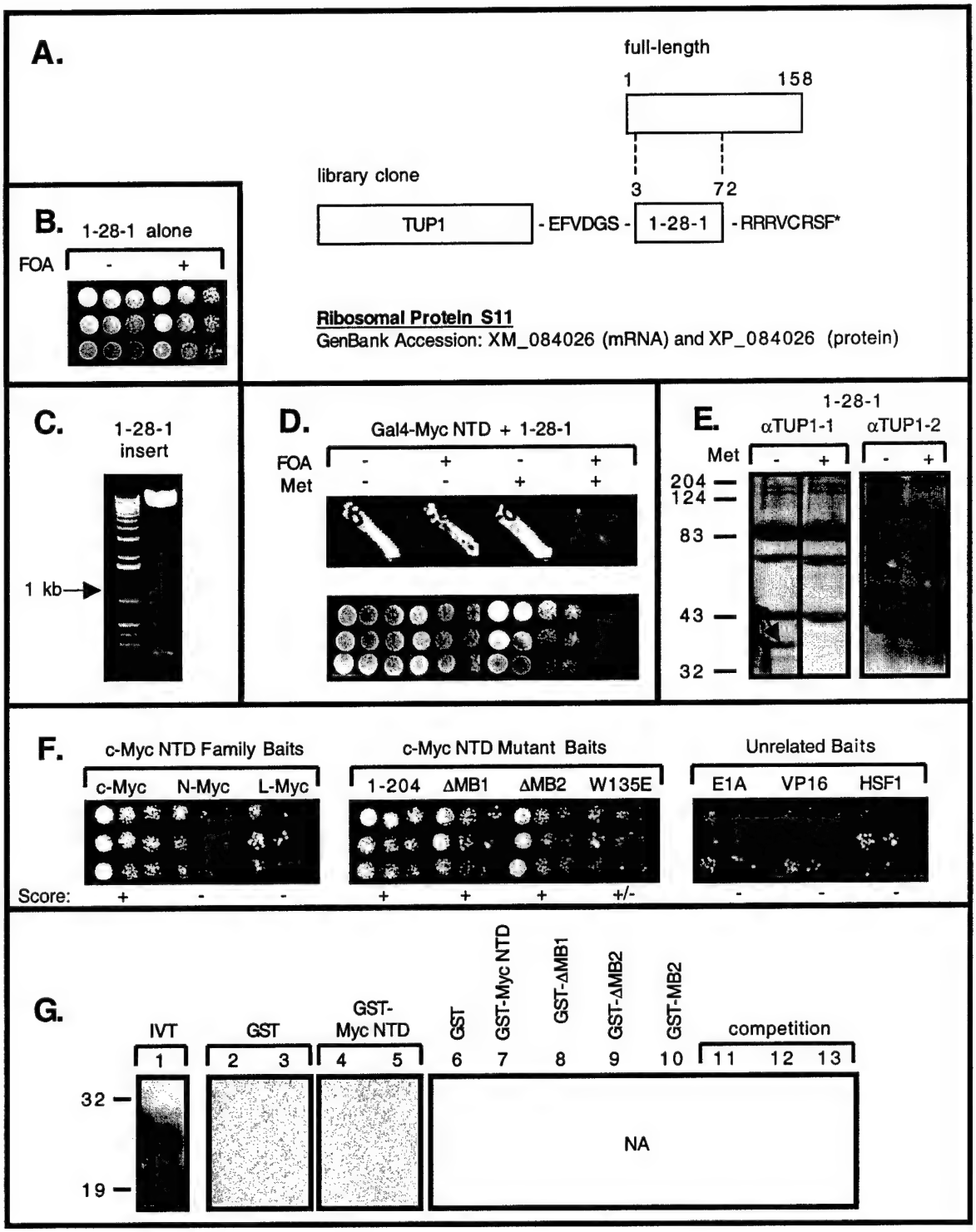


Figure B2. Library clone 1-28-1, Ribosomal Protein S11

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

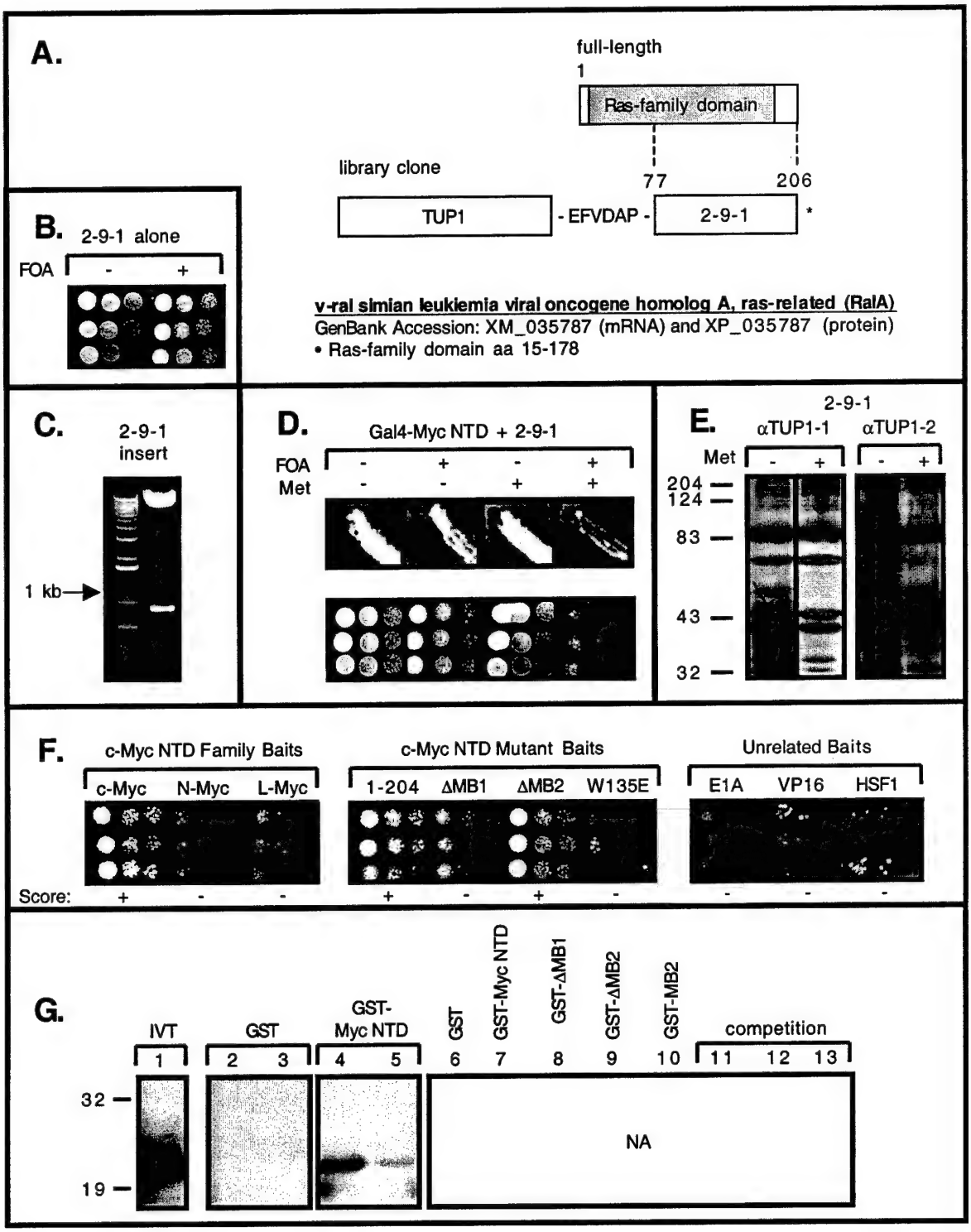


Figure B3. Library clone 2-9-1, v-ral simian leukiemia viral oncogene homolog A, ras-related (RalA)
(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

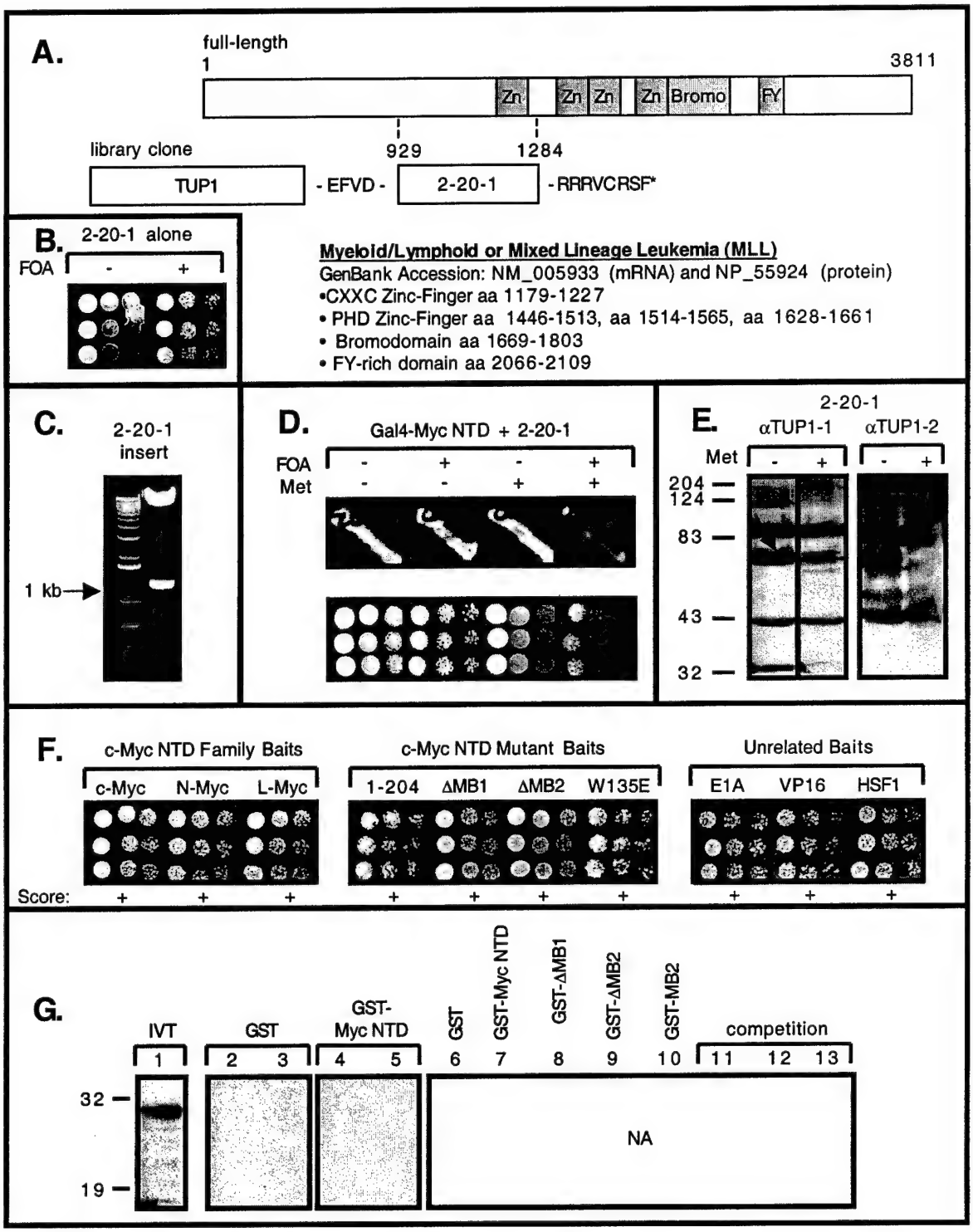


Figure B4. Library clone 2-20-1, Myeloid/Lymphoid or Mixed Lineage Leukemia (MLL)
(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

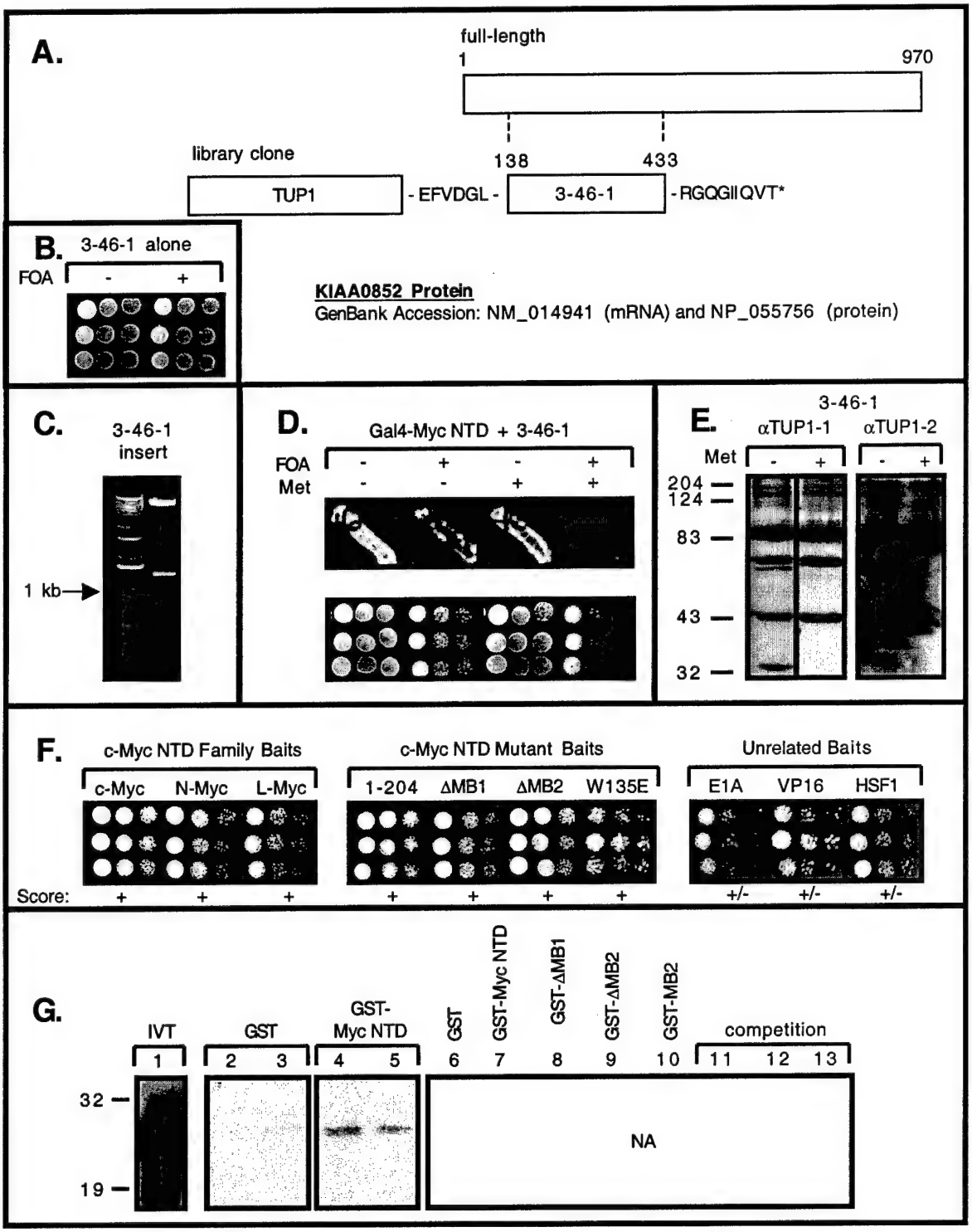


Figure B5. Library clone 3-46-1, KIAA0852 Protein

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

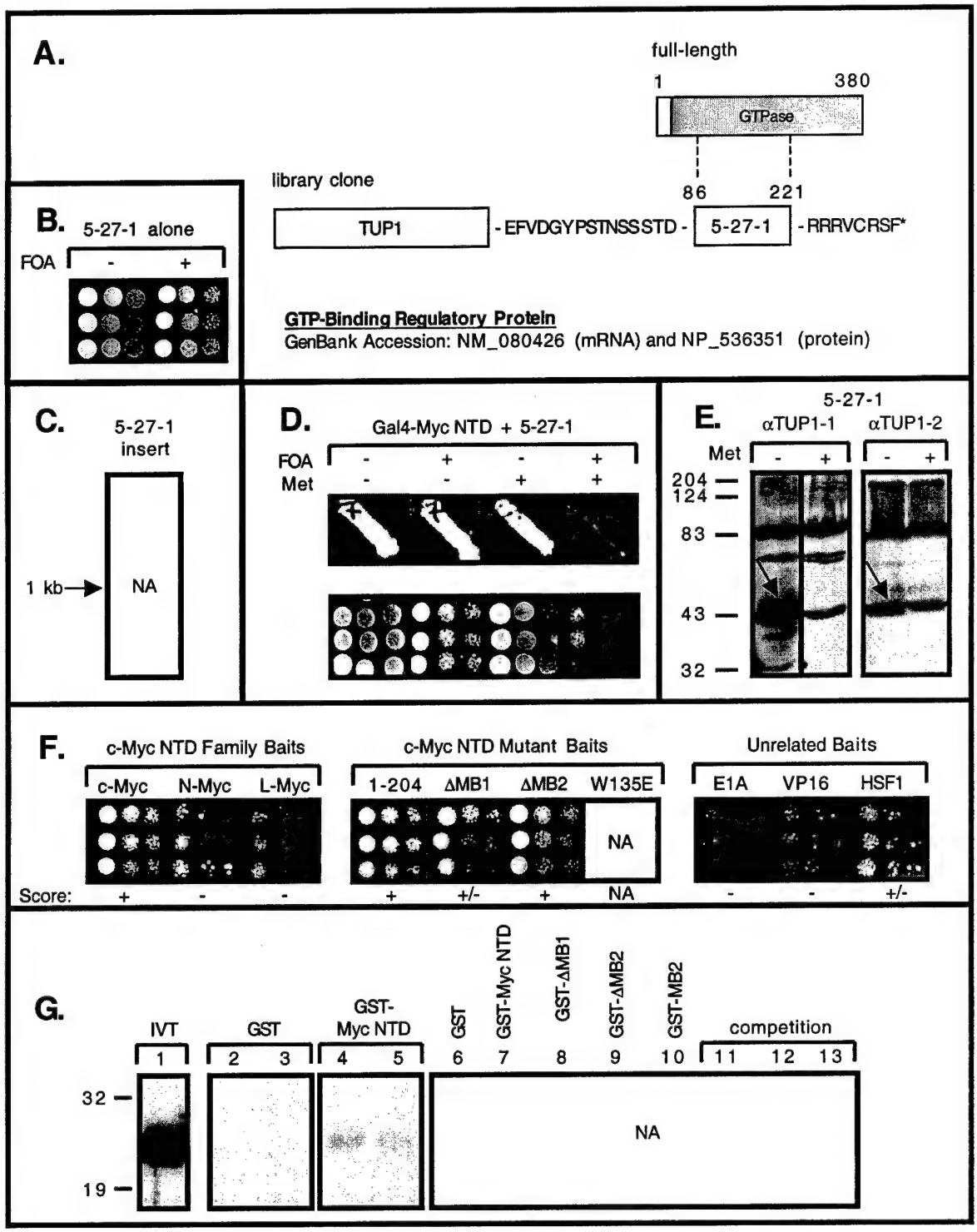


Figure B6. Library clone 5-27-1, GTP-binding regulatory protein

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

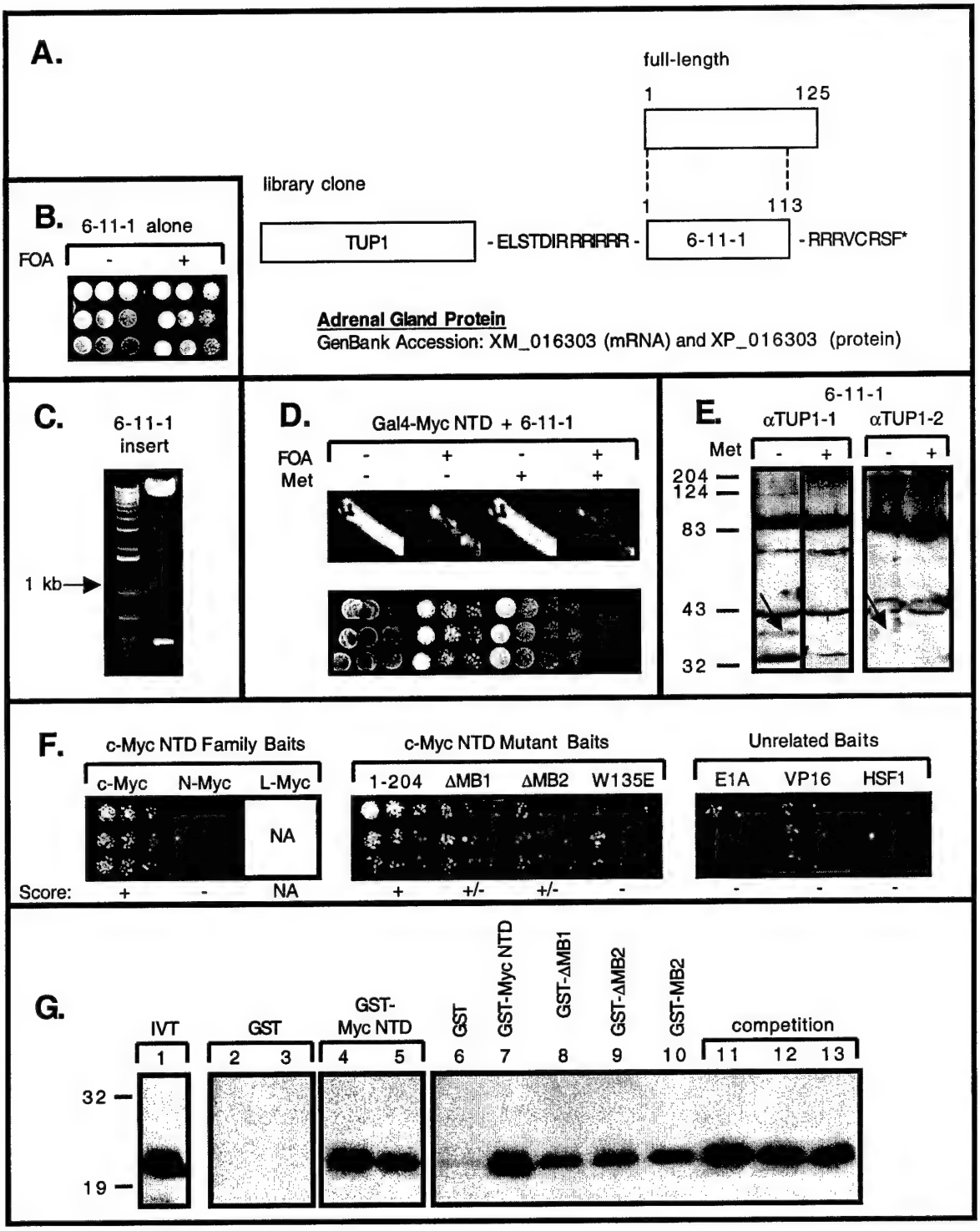


Figure B7.Library clone 6-11-1, Adrenal Gland Protein

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

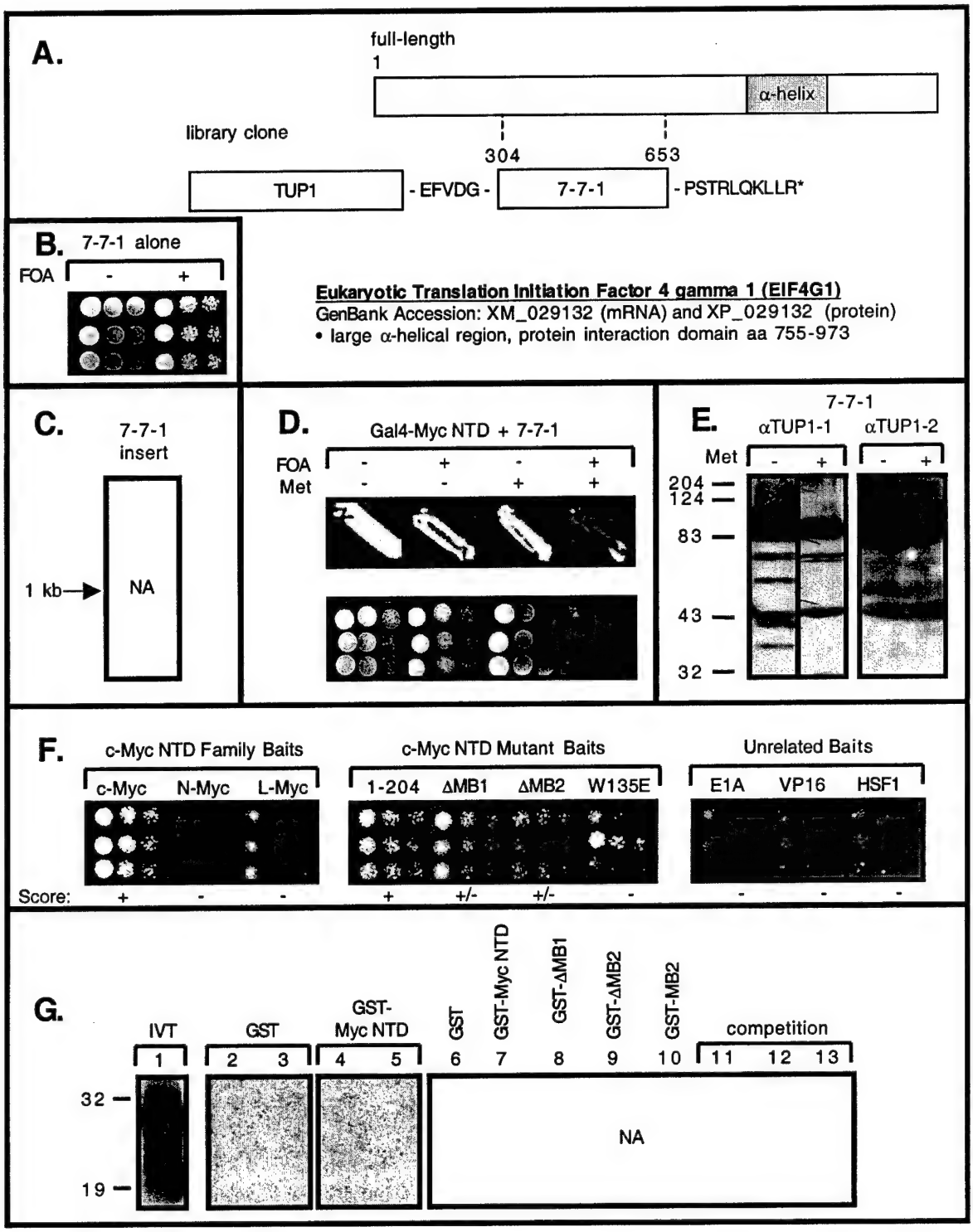


Figure B8. Library clone 7-7-1, Eukaryotic Translation Initiation Factor 4 gamma 1 (EIF4G1)
(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

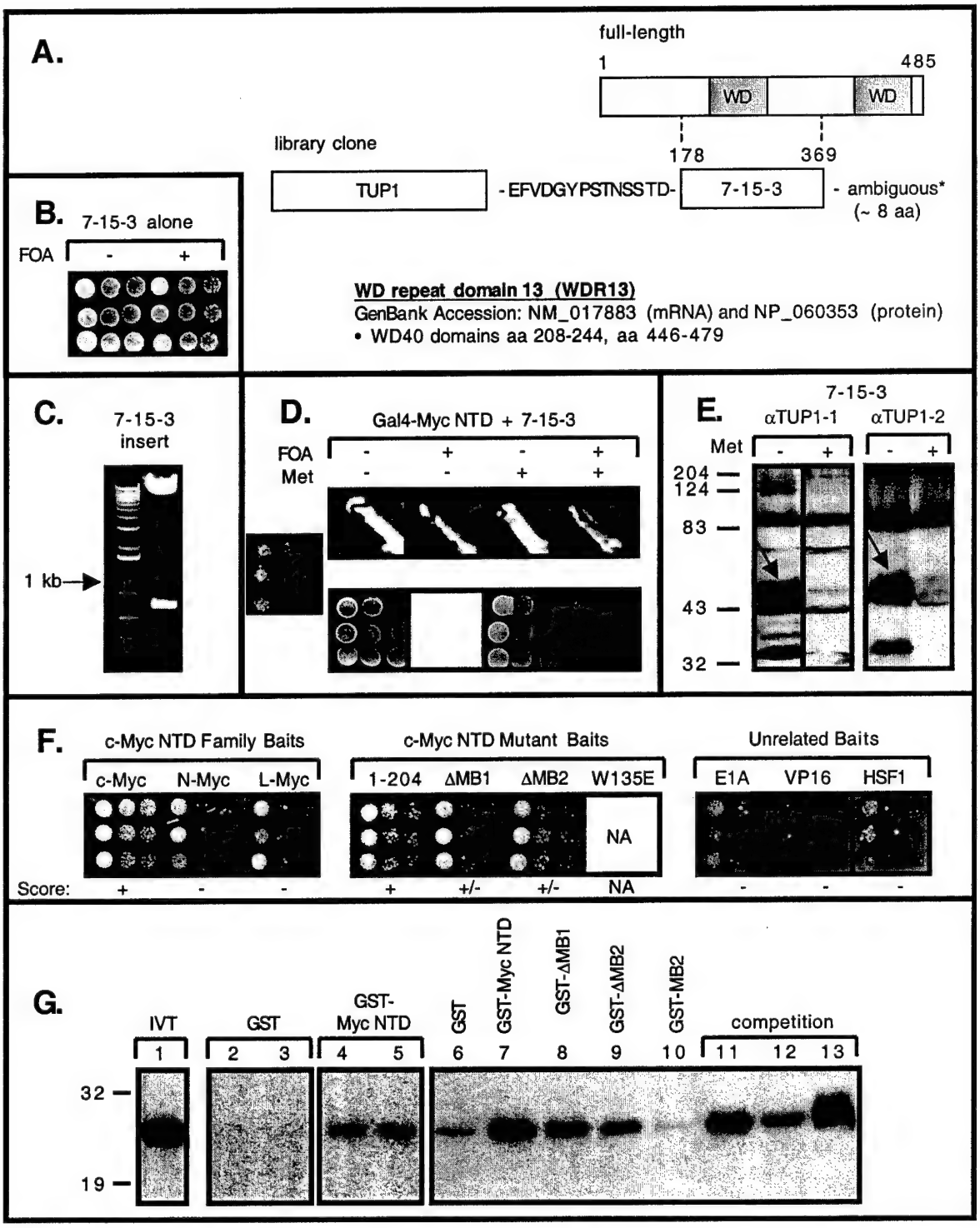
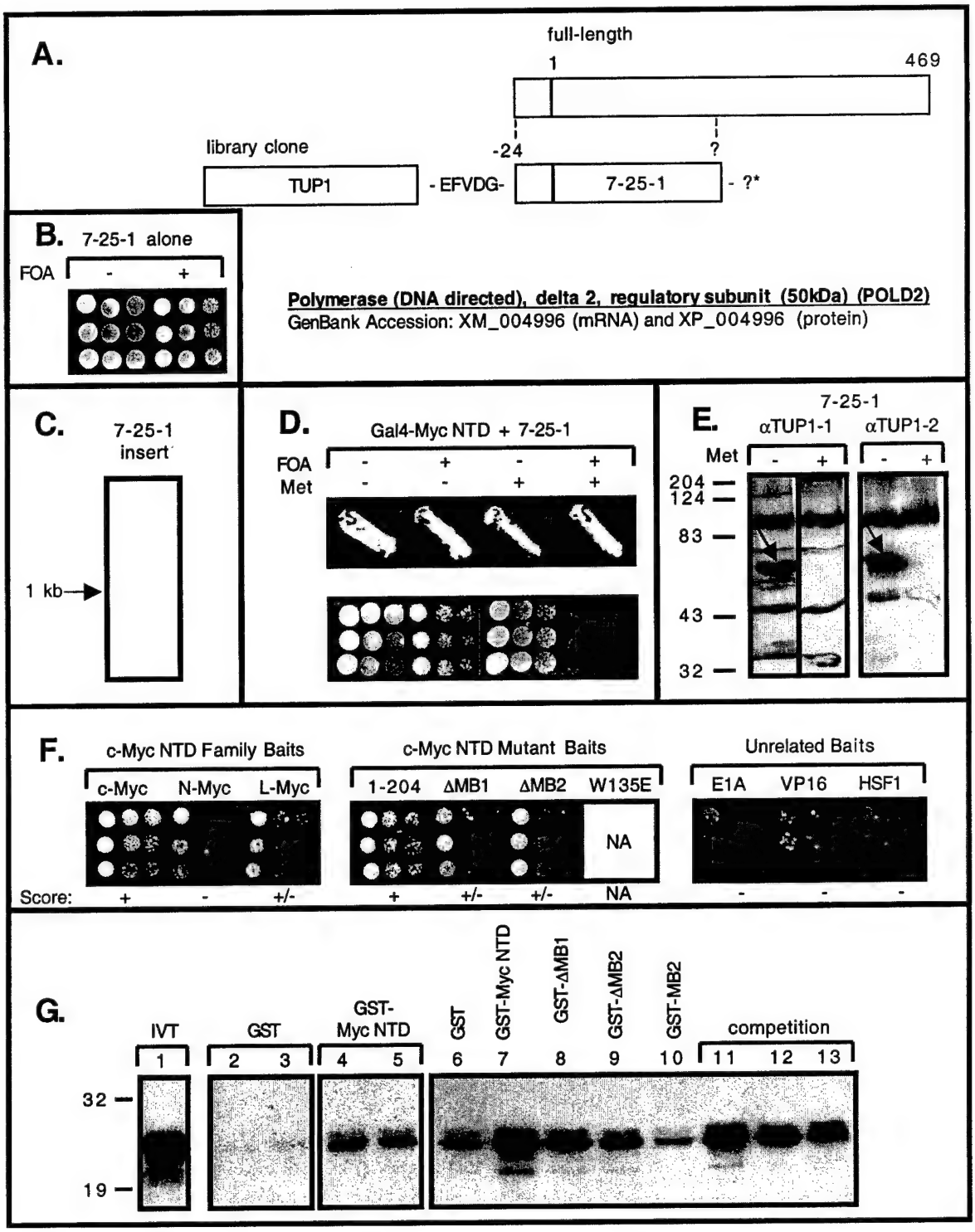


Figure B9. Library clone 7-15-3, WD repeat domain 13 (WDR13)

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).



Appendix C.10 Library clone 7-25-1, Polymerase (DNA-directed), delta 2 (50kDa) (POLD2)

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

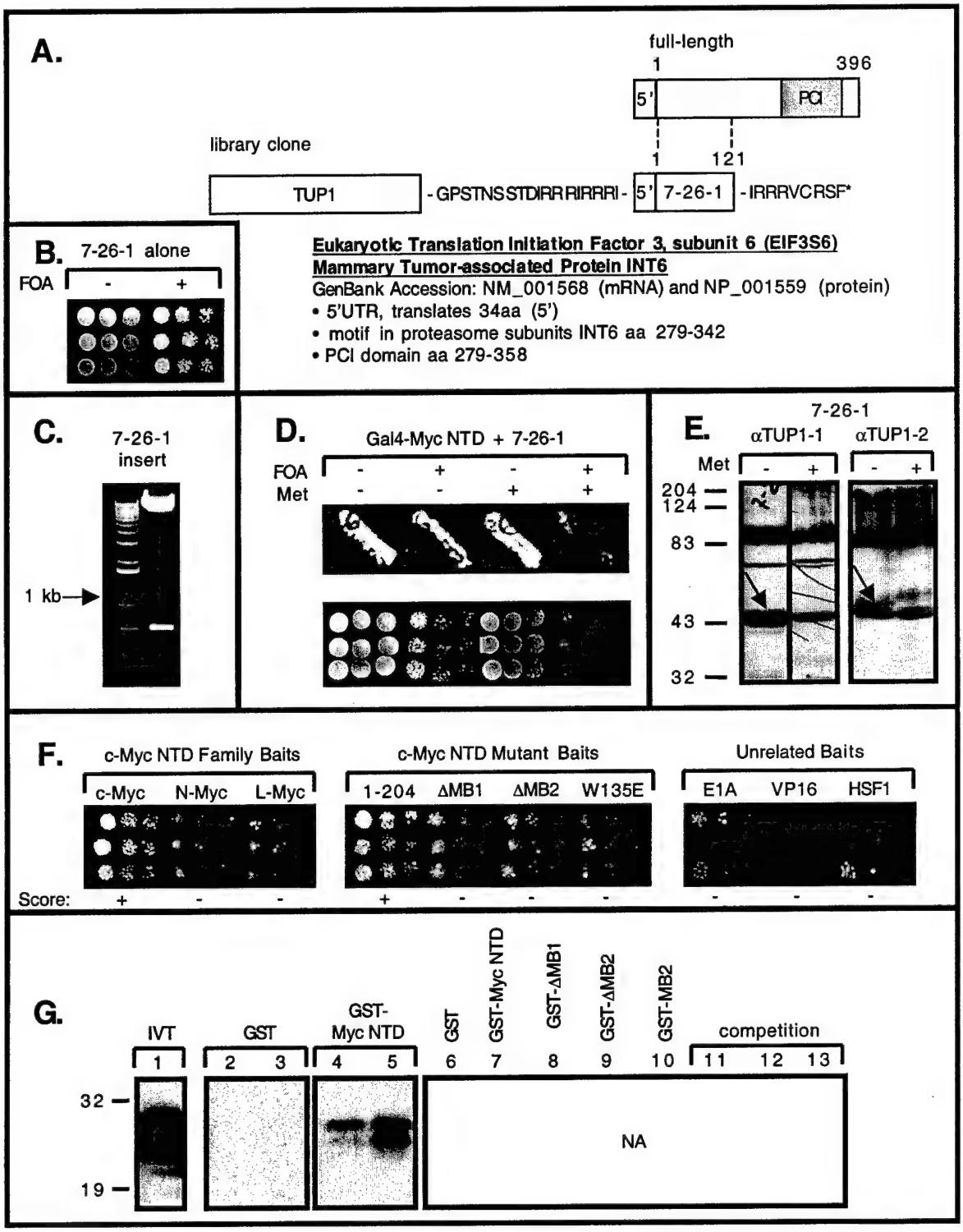


Figure B11. Library clone 7-26-1, Mammary Tumor-associated Protein INT6

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

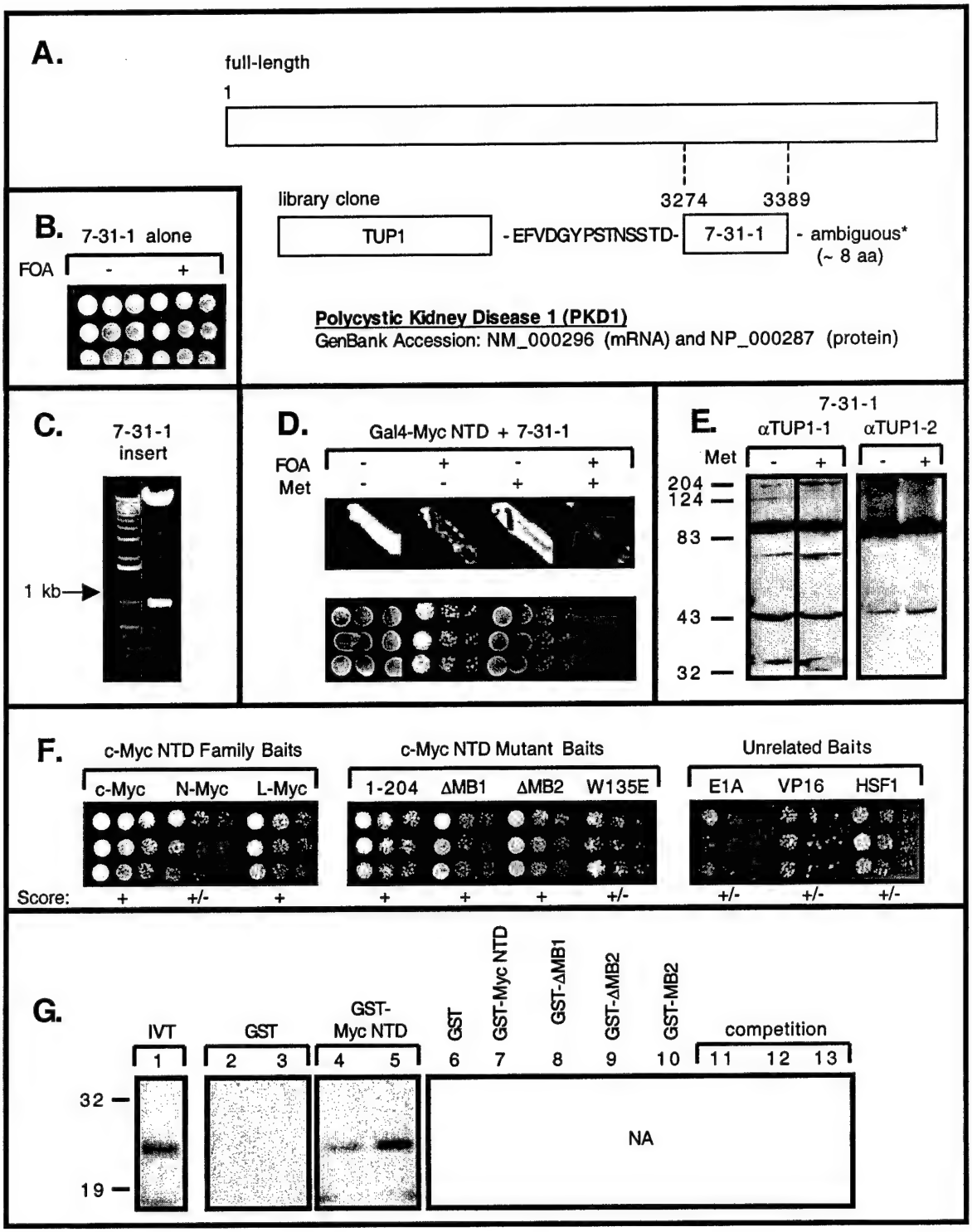


Figure B12. Library clone 7-31-1, Polycystic Kidney Disease 1 (PKD1)

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

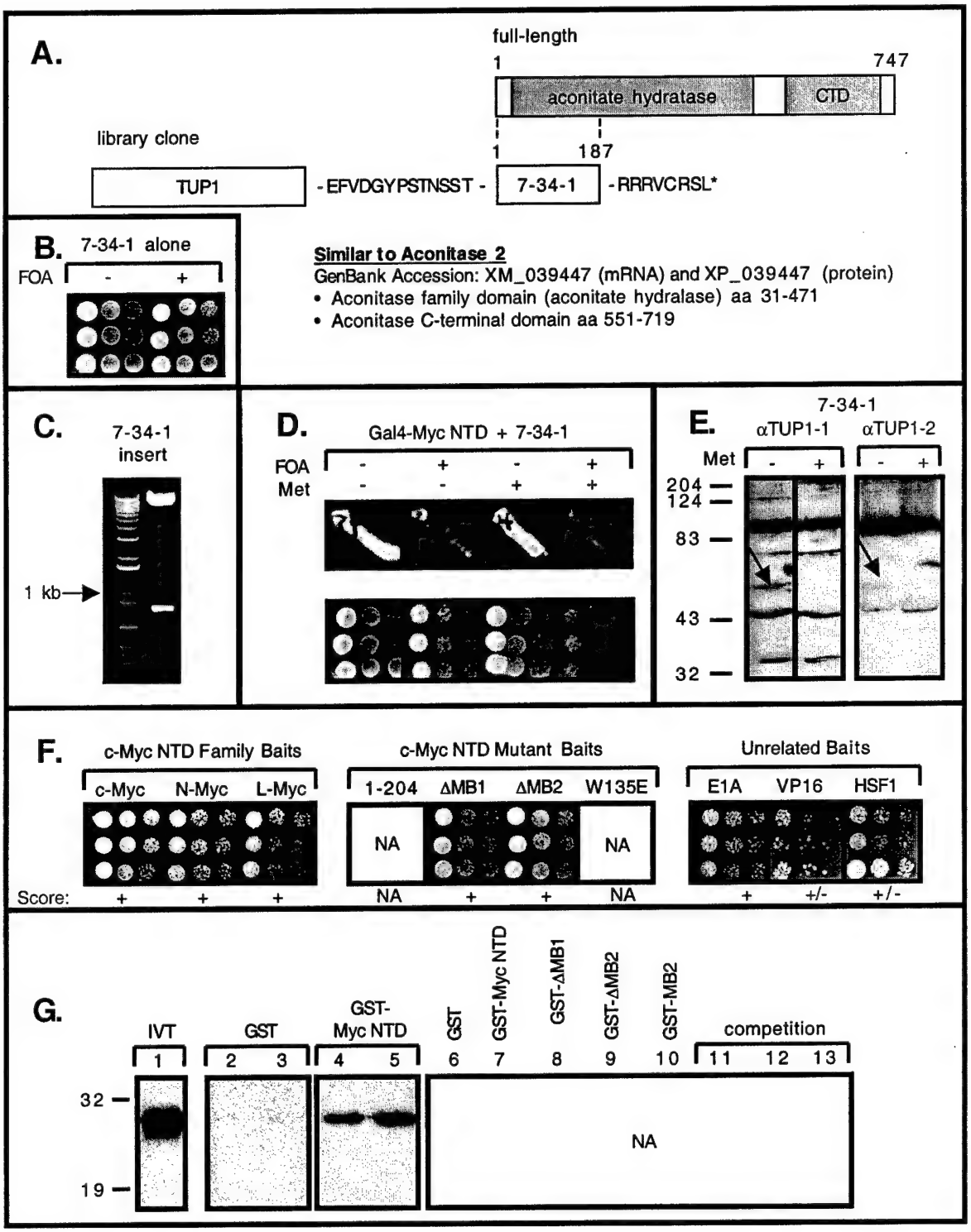


Figure B13. Library clone 7-34-1, Similar to Aconitase 2

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

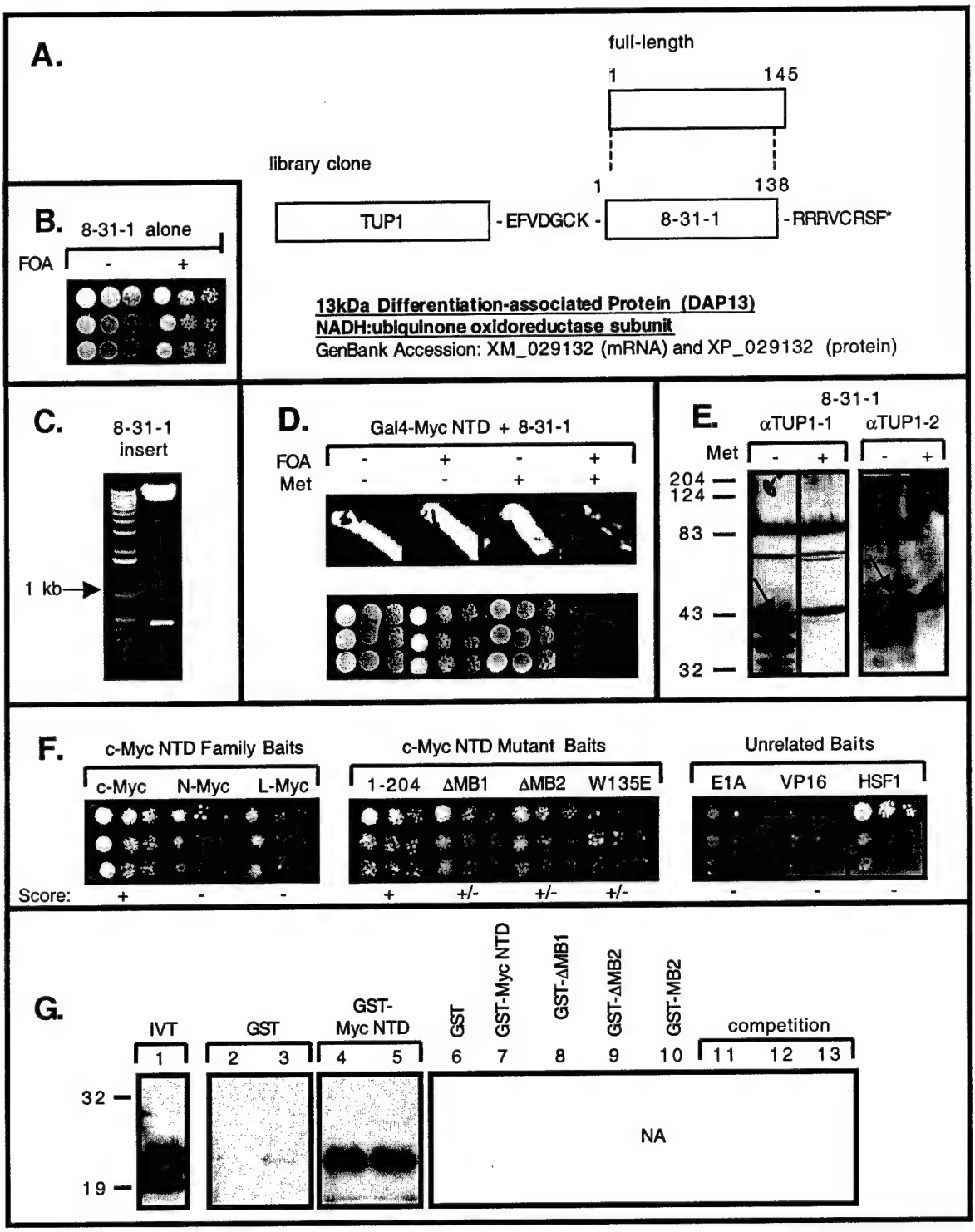


Figure B14. Library clone 8-31-1, 13kDa Differentiation-associated Protein (DAP13)
(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

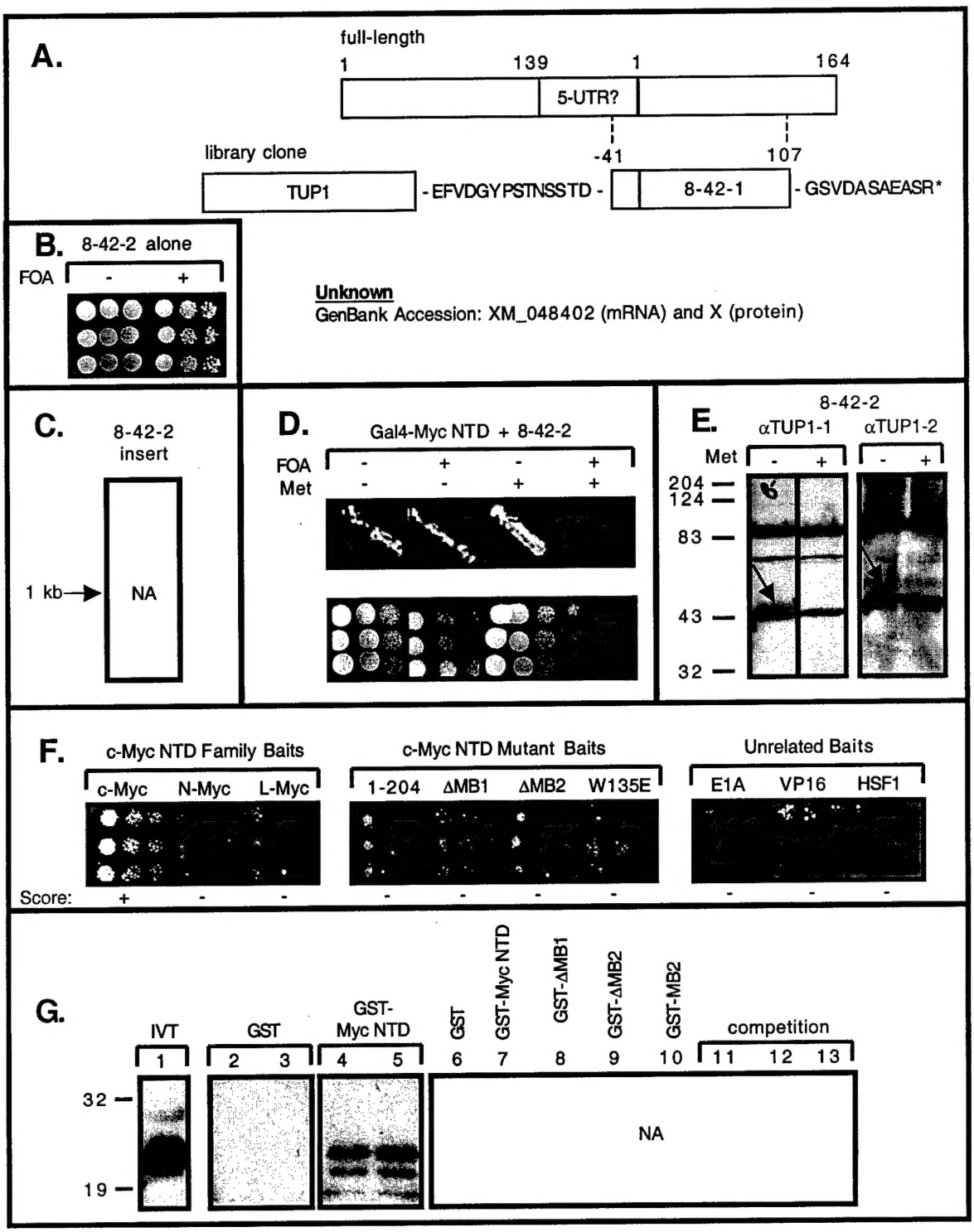


Figure B15. Library clone 8-42-2, Unknown

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

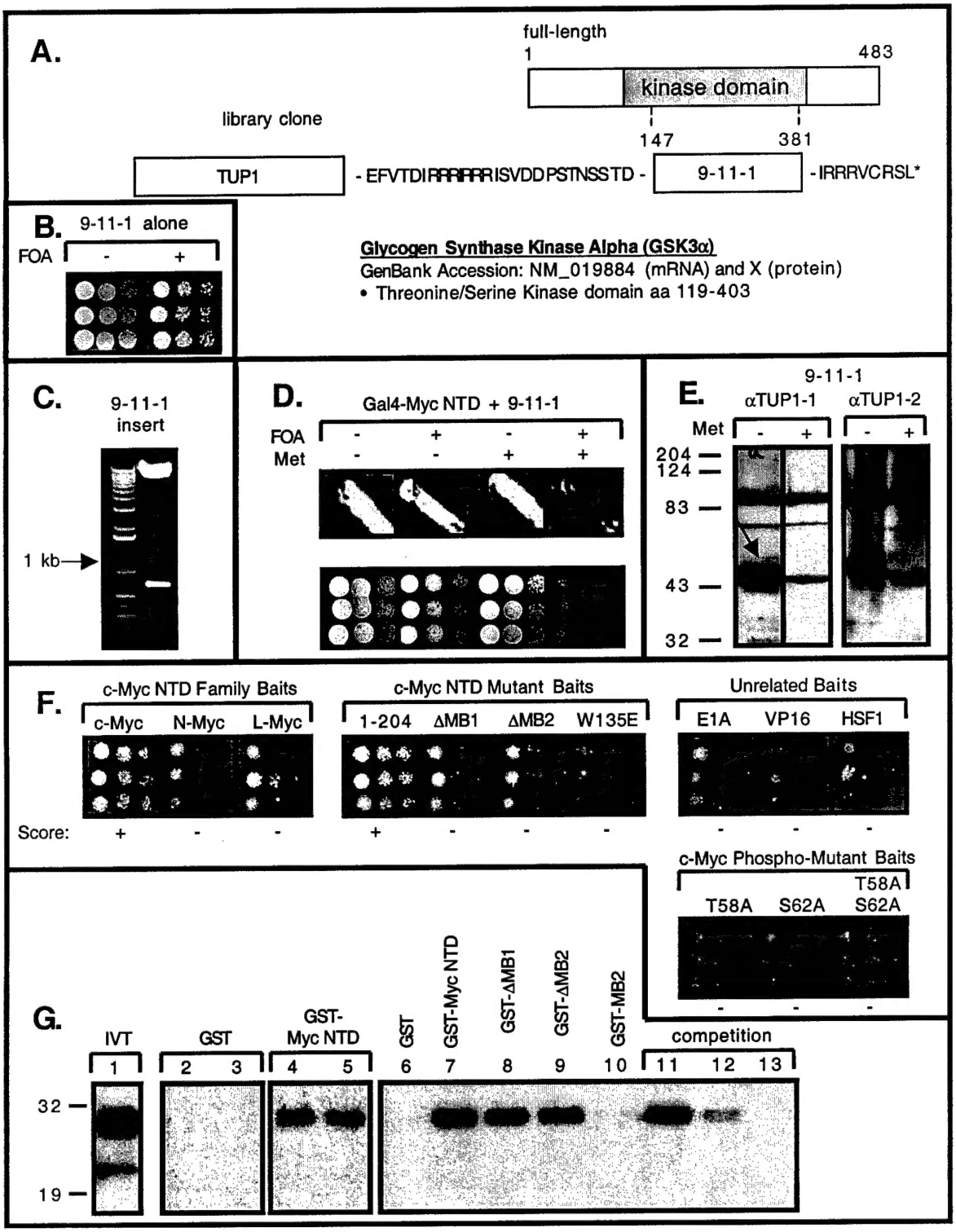


Figure B16. Library clone 9-11-1, Glycogen Synthase Kinase Alpha (GSK3α)

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

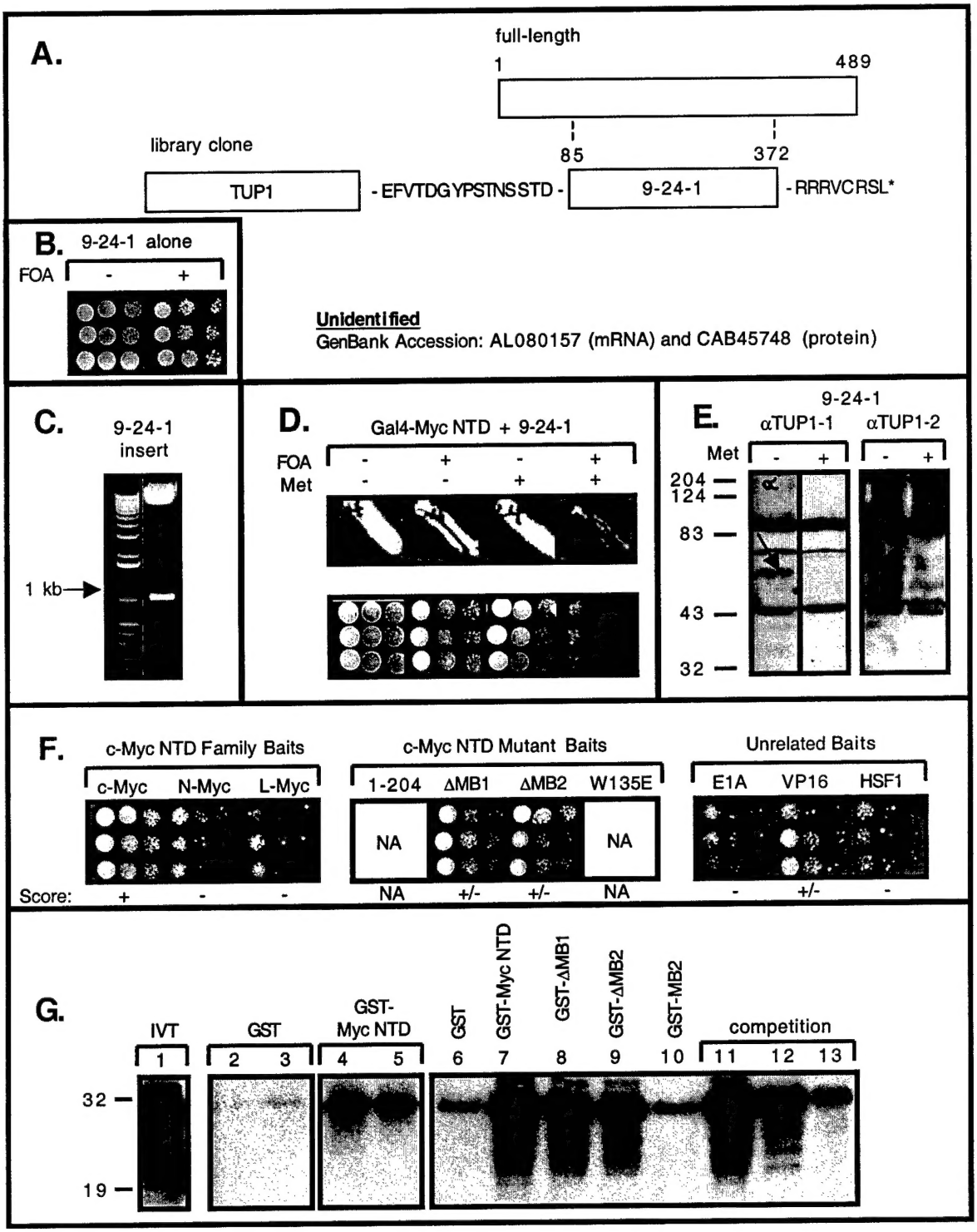


Figure B17. Library clone 9-24-1, Unidentified

(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).

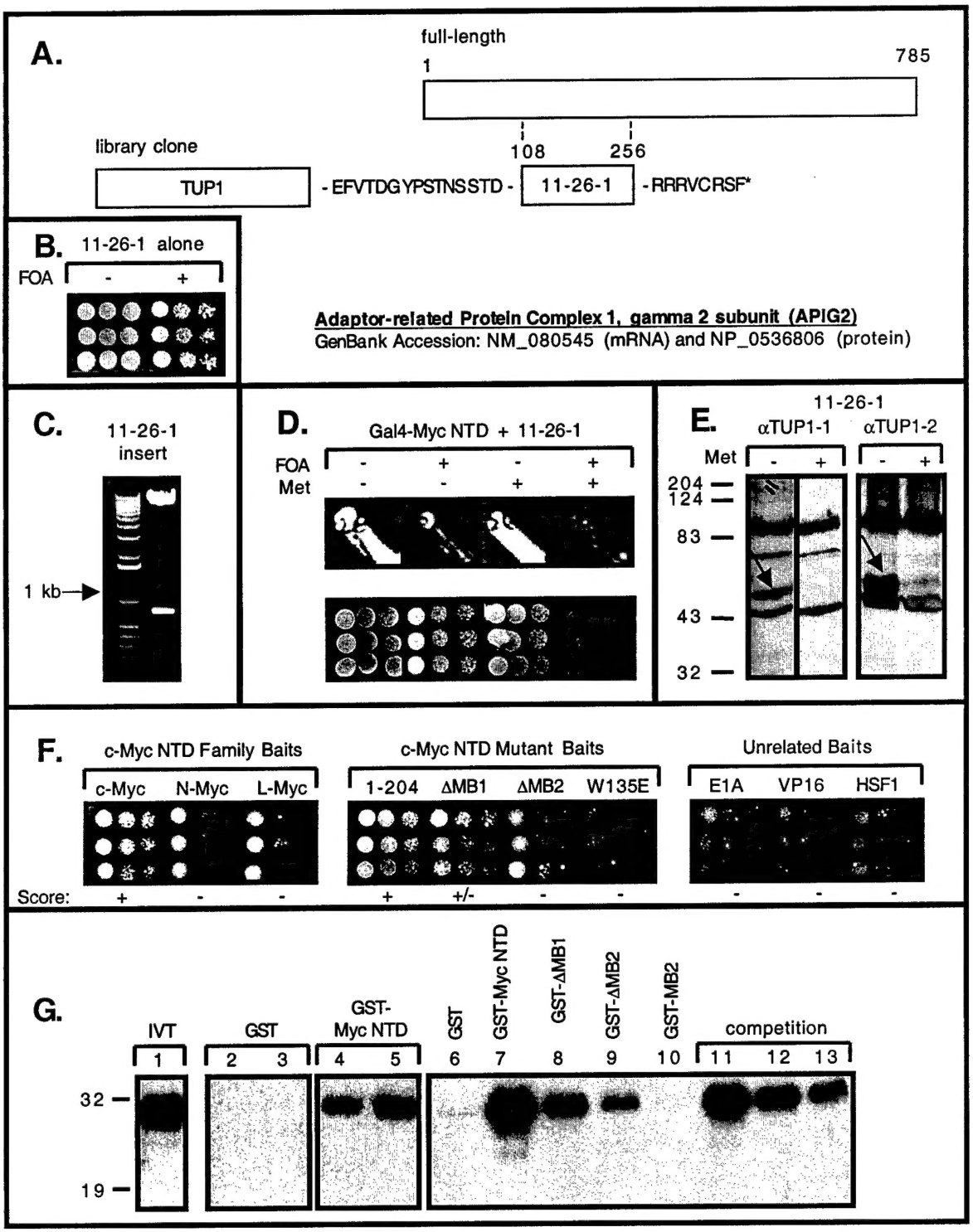


Figure B18. Library clone 11-26-1, Adaptor-related Protein Complex 1, gamma 2 subunit (APIG2)
(A) Schematic representation of the TUP1-fusion library clone against the full-length protein sequence **(B)** Expression of the library clone alone on +/-FOA **(C)** EcoRI/BamHI restriction digest to release cDNA insert from pBDH vector **(D)** Original selection on +/-FOA,+/-Met (upper panel); Retransformation of library clone with Gal4-Myc NTD bait into fresh yeast cells on +/-FOA,+/-Met media (lower panel) **(E)** αTup1 immunoblot analysis of Tup1-fusion expression under +/-Met conditions, specific bands marked by an arrow **(F)** Coexpression of library proteins with Myc-related and -unrelated activator baits on +FOA, score is relative to growth with c-Myc **(G)** *In vitro* translation (IVT) of library protein, binding experiments using crude lysates of recombinant GST-fusion proteins (lanes 2-10) and competition experiments using purified GST-free Myc NTD (lanes 11-13).